The potential of laser-induced fluorescence (LIF) spectra of sheep feces to determine diet botanical composition

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Abstract

Laser-induced fluorescence (LIF) peaks near 470 nm ('blue') and 650 nm ('red') from sheep feces treated with chloroform ($CHCl_3$) displayed spectral signatures that varied with diet. Fecal samples were obtained rectally over 3 days in a replicated study consisting of four diets (treatments) fed to 16 Polypay and Polypay × Rambouillet lambs (four lambs per treatment). Basal diet consisted of tobosa (*Pleuraphis mutica* Buckley [formally *Hilaria mutica* (Buckl.) Benth.] hay with 0% (control), 10%, 20% or 30% tarbush (*Flourensia cernua* D.C.) added on a dry matter basis. Feces were dried at 60°C, treated with chloroform and the extract from both crushed and intact fecal material was then excited by laser input at 355 nm. Tarbush leaves gave LIF only in the blue region of the spectrum. However, tobosa hay and all fecal pellets produced a bimodal LIF spectral distribution with a lower, broader peak feature in the blue region and a narrower well defined taller feature in the red region of the spectrum. Chloroform did not exhibit fluorescence peaks between 400 nm and 800 nm. As tarbush in the diet increased, so did the magnitude of counts in the blue region of the spectrum. The LIF red/blue intensity ratios appear to be more reliable than actual counts in differentiating feces of sheep fed tobosa diets containing differing amounts of tarbush. Red/blue ratios decreased ($P < 0.05$) linearly ($P < 0.005$) for both crushed and intact sheep fecal pellets as the tarbush/tobosa ratio in the diet increased. Inconsistencies in count amplitudes and wavelengths in either one or both regions (red or blue) of the spectrum and in red/blue ratios (especially on Day 3 for crushed fecal samples) are discussed in terms of sample preparation procedures and sampling days. Our results suggest that further testing of the LIF technique is warranted using diets consisting of several species.

Keywords: Diets; Sheep; Fecal samples; Laser-induced fluorescence (LIF); Botanical composition

1. Introduction

There is tremendous need for a fast, accurate and non-invasive procedure to evaluate free-ranging animal diets. Currently, diet botanical composition is

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estimated by: (1) Directly observing what an animal consumes; (2) Sampling digesta from inside the gastrointestinal tract; or (3) Sampling the digesta post-excretion. These methods all have limitations (Holechek et al., 1984).

Method three is predominantly used to quantitatively estimate diets of free-ranging animals, especially when handling animals is not possible (Dearden et al., 1975). In addition, fecal samples represent a broad spatial and temporal range of dietary intake and are suitable for approximating diet selection on a large scale (Norbury and Sanson, 1992). In the earliest study of feces components, rabbit diets were evaluated (Dusi, 1949). Later, the technique was used with sheep in New Zealand (Croker, 1959). Today, when combined with the microhistological technique, evaluating feces may be the most widely used method for estimating range herbivore diets (Holechek et al., 1982). Epidermal characteristics form the basis for plant fragment identification (Davies, 1959). Feces contain relatively small fragments that are resistant to mechanical and chemical digestive processes; however, few of them can be recognized without magnification (Ward, 1970). Variable digestibility among plants and plant parts appears to be the most serious source of error in determining diet composition from fecal examination (Regal, 1960).

Therefore, replacing the microhistological technique with a more rapid and less tedious procedure to accurately estimate diets of free-ranging animals continues to be a research focus. Current alternatives to microhistological analysis include infrared reflectance (Shenk et al., 1978; Coleman et al., 1985), the use of pinitol (Forwood et al., 1987), calcium content (Playne et al., 1978), the ratio of ^{13}C and ^{12}C isotopes (Jones et al., 1979) and the use of n-alkanes in plant cuticular wax (Dove and Mayes, 1991); however, all have limitations.

We explored the use of laser-induced fluorescence (LIF) for monitoring animal diets. Fluorescence is the re-emission of light by a material that has been excited by incident light; in LIF, the excitation source is emission from a laser. Typically the re-emitted light has a longer wavelength than the excitation light. The spectrum of fluorescence is characteristic of the fluorescing material and, hence, can serve for identification. A material's fluorescence can also

depend upon the spectral character of the exciting light. All objects will fluoresce when excited with the proper wavelength (Brach et al., 1977).

Fluorescent properties of plant pigments have been documented for well over 100 years (Rabinowitch, 1956). Hickman and Moore (1970) were the first to describe LIF of chlorophyll a in algae. Relatively little research has been done on the spectral properties of intact plants (Gates et al., 1965). However, Brach and Molnar (1977) successfully used remote spectroscopic techniques to identify and determine maturity of horticultural crops including spring onion, pea, radish and lettuce. The LIF of other terrestrial plants (Boutton and Tieszen, 1983; Hoge et al., 1983; Lichtenthaler et al., 1993), and algae and phytoplankton (Kim, 1973; Hoge and Swift, 1983) have also been investigated. Recently, Ourcival et al. (1992) used fluorescence to rank genotypes within white clover (*Trifolium repens* L.) and perennial rye grass (*Lolium perenne* L.).

However, a review of the literature indicates that none of the studies involving fluorescence of vascular plants have focused on those commonly eaten by free-ranging ruminants on arid rangeland or on digested plant material (Gates et al., 1965; Kim, 1973; Brach et al., 1977; Hoge et al., 1983; Chappelle et al., 1984a, Chappelle et al., 1984b, Chappelle et al., 1985 and Chappelle et al., 1991; Stober and Lichtenthaler, 1993). The objective of our study was to determine if feces obtained from pen-fed sheep receiving a basal diet of tobosa (*Pleuraphis mutica* Buckley [formerly *Hilaria mutica* (Buckl.) Benth.] hay with 0%, 10%, 20% or 30% tarbush (*Flourensia cernua* D.C.) leaves would produce distinguishable LIF spectra.

2. Materials and methods

2.1. Animals, diets and fecal samples

Tarbush plants were harvested from the Jornada Experimental Range and air dried; then leaves were removed. Mature tobosa grass was mowed, air dried and ground through a 2.54 cm screen in a New Holland model 358 mix mill. Chemical composition of the tobosa hay and tarbush leaves, expressed on a dry matter basis, was 6% and 19% CP (crude pro-

tein) and 80% and 33% NDF (neutral detergent fiber), respectively, (King et al., 1996). Four experimental diets (treatments) were evaluated: mature tobosa hay with 0% (control), 10%, 20% or 30% whole tarbush leaves added on a dry matter basis. Rectal grab fecal samples were obtained from 16 ruminally cannulated Polypay and Polypay × Rambouillet lambs (four lambs per treatment) having a mean liveweight of 46 ± 1.6 kg. Feces were collected for three consecutive days immediately following a companion pen feeding trial (King et al., 1996). Lambs were individually fed (2.4×4.7 m pens) diets twice daily at 07:00 and 16:00 h for 29 days prior to fecal collections. Water and a trace mineral block were provided ad libitum and orts were weighed before each morning feeding with minimal separation of diet components by the sheep among treatments (King et al., 1996).

Fecal samples were collected daily at 11:00 h and immediately placed in plastic bags and frozen. Two fecal pellets from each sheep from all 3 days ($n = 16 * 2 * 3$) and two more fecal pellets from Days 2 and 3 ($n = 16 * 2 * 2$) were thawed and oven dried to a constant weight (0.0001 g) at 60°C. Individual pellets, each weighing approximately 0.32 g, were then placed in 160 vials each containing 20 ml of HPLC grade chloroform ($CHCl_3$), capped, and maintained at room temperature (19–24°C) until pellets sank to the bottom of the vial.

Individual pellets in 96 of the vials were crushed with forceps, and three 4 mm diameter glass beads were placed in each vial. The contents were agitated on a vortex mixer until no particles larger than the glass beads were visible (typically 2–3 min). The pulverized material was then poured into a 100 ml beaker and filtered through Whatman No. 4 paper. The 100 ml beaker was rinsed with an additional 5 ml of $CHCl_3$ to remove all particles. The filtrate (extract) was re-captured in the original vials and the vials were recapped. Pellets were removed from the remaining 64 vials and recapped to prevent $CHCl_3$ evaporation. (The two methods of pellet preparation ['crushed' and 'intact'] were used to determine if the more rapid non-crushing procedure would produce similar results to the more involved procedure requiring crushing and filtration.) The additional 5 ml of solvent used with the crushed samples likely reduced LIF signal strengths by dilution, without affecting

spectral shapes. Therefore, data from the two preparation techniques were analyzed separately.

Six vials each of filtrate from the two diet components were also prepared for LIF measurements by placing either tobosa hay (approximately 10 g) or ten intact tarbush leaves in a beaker with 20 ml of $CHCl_3$, swirling the mixture for 20 s and then filtering as with the crushed fecal pellets. Another six vials containing only $CHCl_3$ served as blanks. All 178 capped vials were placed in a freezer ($-20°C$) until tested for LIF.

2.2. Laser procedure

The 160 vials of $CHCl_3$ exposed to fecal samples were tested in random order for LIF by pipetting 3 ml aliquots from each vial into a 3.5 ml quartz cuvette placed in a holder in the laser beam. Following exposure to the laser beam, the material in the cuvette was returned to its original vial, the cuvette was rinsed with pure HPLC grade $CHCl_3$, and the wash was discarded. The $CHCl_3$ blanks, tobosa hay filtrate, and tarbush leaf filtrate were evaluated at the beginning and end of the LIF testing and after every 30 to 33 fecal samples.

Each sample in the cuvette was excited by a series of four pulses of 355 nm light from a frequency-tripled neodymium:yttrium aluminum garnet (Nd:YAG) laser (Model DCR-2A Quanta-Ray manufactured by Spectra-Physics). The pulses, spaced by 0.1 s, had durations of about 10 ns and energies of about 1 mJ. The resulting broadband fluorescence was input to a low resolution spectrograph (Model HR 320 manufactured by Instruments SA, with 150 groove mm^{-1} holographic grating). Two long-pass color filters were used at the spectrograph input to keep bright scattered laser light from entering the instrument and possibly distorting the fluorescence spectrum. The dispersed spectrum at the spectrograph's output was detected pulse-by-pulse using an intensified time-gated linear photodiode array (Model TN-6144 manufactured by Tracor Northern). Output of the array, whose 774 central pixels covered the wavelength range from 400 to 800 nm, was stored in a computer. The resulting spectra consisted of 'counts' (proportional to fluorescence light intensity) plotted against pixel number (corresponding to wavelength).

2.3. Data procedures

The data analyzed consisted of subsample mean values of maximum counts in the blue and red and their corresponding wavelengths. The mean ($n = 6$ samples with four laser pulses per sample) spectrum of the $CHCl_3$ only was considered to be the baseline and was subtracted from each of the four laser pulses per sample (774 count-wavelength pairs produced per pulse) before selecting the maximum red and blue count and corresponding wavelength. For each sample the resulting peak count values, corresponding wavelengths and red/blue count ratios, based on the subsample mean ($n = 4$ laser pulses per sample) were analyzed statistically.

Data for the crushed and intact pellets were analyzed using a split plot where time is day. The whole plot (pellet) analysis was a Completely Randomized Design with diet as the whole plot factor and animal within diet as 'error a'. Linear, quadratic and cubic contrasts were tested when the diet F-test was significant ($P \leq 0.05$). Analyses were performed using the GLM procedure (Statistical Analysis Systems Inc., 1989). Least square means and standard errors were calculated for diet, day and the diet × day interaction. The PDIFF option (similar to LSD) was used to separate day means when the overall day F-test was significant at $\alpha = 0.05$.

3. Results

Tobosa hay produced two LIF features with the blue feature peaking between 451 and 466 nm and a distinctive red feature at approximately 675 nm (Fig. 1). In contrast, tarbush leaves exhibited only a blue feature which peaked between 450 and 463 nm (Fig. 1). Pure $CHCl_3$ did not exhibit fluorescence peaks between 400 and 800 nm. All fecal pellets produced two distinctive peaks similar to tobosa, with most samples showing a lower and broader blue feature compared to a taller and more distinct red feature (Fig. 2).

Except for the blanks with $CHCl_3$ only, all the remaining 172 samples produced LIF features in either the blue or blue and red regions of the spectrum. Crushed fecal pellets from different diets differed quantitatively (fluorescence amplitude) and
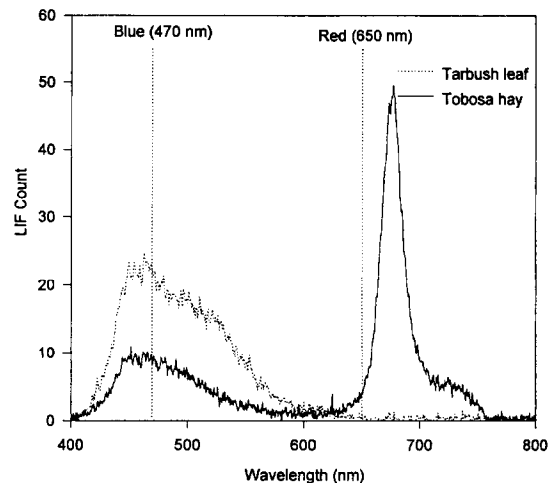

Fig. 1. Raw mean counts ($n = 6$) from laser-induced fluorescence (LIF) between 400 and 800 nm for chloroform ($CHCl_3$) extract from mature tobosa (*Pleuraphis mutica* Buckley) hay and intact tarbush (*Flourensia cernua* D.C.) leaves having maximum count standard errors of ± 3.4 and ± 1.9 counts, respectively.

qualitatively (wavelength) in the blue but not the red. Except for the blue wavelength, which did not differ across diets, the same relationship was also true for intact pellets. The red/blue count ratio was used to
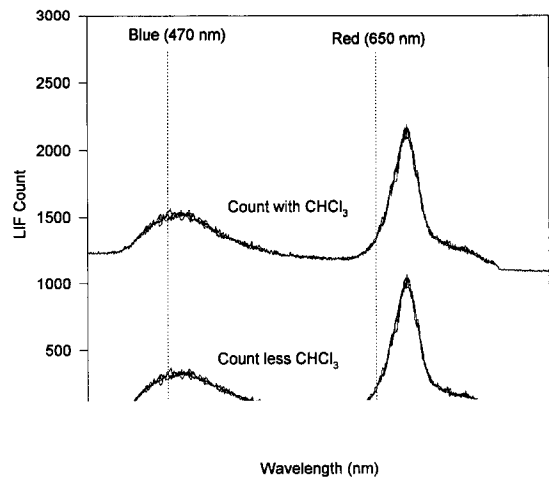

Fig. 2. Laser-induced fluorescence (LIF) amplitude (counts) between 400 and 800 nm resulting from exciting the chloroform ($CHCl_3$) extract from a single crushed sheep fecal pellet with a series of four pulses of 355 nm light. Prior to collecting the fecal material the sheep had been fed a diet containing 70% tobosa (*Pleuraphis mutica* Buckley) hay and 30% tarbush (*Flourensia cernua* D.C.) leaves for 29 days.
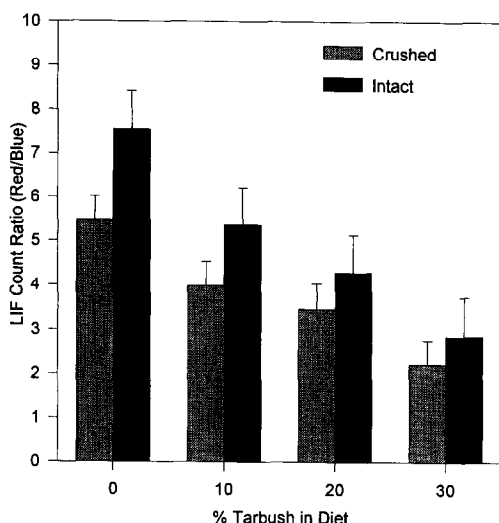
Fig. 3. LsMean ± standard errors for red to blue laser-induced fluorescence (LIF) count ratios from crushed and intact fecal pellets extracted in chloroform ($CHCl_3$) from sheep fed a basal tobosa (*Pleuraphis mutica* Buckley) hay diet containing 0%, 10%, 20% or 30% tarbush (*Flourensia cernua* D.C.) leaves. Feces from the four diets differed ($P < 0.05$) linearly ($P < 0.005$).

diets for both methods of preparation. Over the four diets, the blue spectrum peak feature in general was broad while the red region peak feature was narrow and more well defined.

3.1. Crushed pellets

Crushed sheep fecal pellets from diets containing 0%, 10%, 20% or 30% tarbush produced different ($P = 0.0110$) LIF red/blue amplitude ratios. These ratios declined 40% in a linear ($P = 0.0015$) fashion as tarbush in the diet increased from 0 to 30% (Fig. 3). In addition, the fluorescence count ($P = 0.0001$) and peak wavelengths ($P = 0.0005$) in the blue region of the spectrum increased linearly ($P = 0.0001$) with the increase in tarbush (Table 1). Because count data are not as robust in delineating differences as is the red/blue ratio, care should be used when interpreting these statistical differences. However, diets did not ($P > 0.05$) influence LIF in the red region of the spectrum. Mean fluorescence characteristic in this region was 916 counts and 675 nm.

Count amplitude ($P = 0.0017$) and wavelength ($P = 0.0256$) in the blue region of the spectrum varied among collection days. Over all days the diet profiles containing tarbush had greater peak amplitudes and longer wavelengths than the diet without tarbush (Figs. 4 and 5, respectively). The blue amplitude and wavelength profiles of sheep feces from the diet containing 30% tarbush was quite similar on Days 1 and 2, but there was a higher count and a longer wavelength on Day 3. In contrast, the amplitude and wavelength profiles from diets containing 0%, 10%, and 20% tarbush were quite similar across the 3 days. In the red region of the spectrum and for

determine if differences existed among fecal pellets derived from diets containing 0 to 30% tarbush.

Chloroform extracts from tobosa hay and from sheep feces obtained from the four diets had a bimodal LIF spectral distribution between 400 nm and 800 nm, with peak features in the blue and red between 451 to 480 nm and 675 to 676 nm, respectively. For crushed sheep feces, fluorescence in the blue occurred at longer wavelengths as tarbush in the diet increased, but no shift occurred for intact pellets. In contrast, fluorescence in the red was similar across

Table 1

Ls Mean ± standard errors for laser-induced fluorescence (LIF) counts or wavelengths in the blue (470 nm) region of the visible spectrum from chloroform ($CHCl_3$) filtrate (extract) of crushed or intact fecal pellets from sheep fed a basal tobosa (*Pleuraphis mutica* Buckley) hay diet containing 0%, 10%, 20% or 30% tarbush (*Flourensia cernua* D.C.) leaves

Method of fecal pellet preparation	Counts						Wavelength (nm)					
	% Tarbush				P Values		% Tarbush				P Values	
	0	10	20	30	Overall	Linear	0	10	20	30	Overall	Linear
Crushed [a]	197 ± 20.2	251 ± 20.2	292 ± 21.0	396 ± 20.2	0.0001	0.0001	473 ± 0.9	478 ± 0.9	479 ± 0.9	481 ± 0.9	0.0005	0.0001
Intact [b]	155 ± 33.7	236 ± 33.7	274 ± 33.7	355 ± 33.7	0.0092	0.0012						

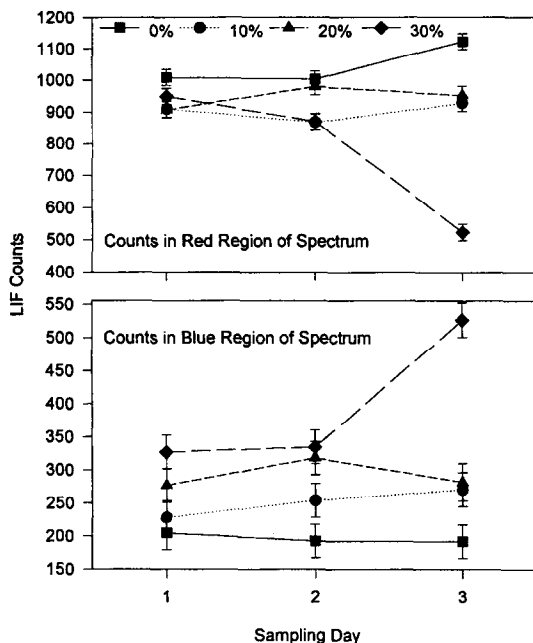[a] Based on three consecutive days.

[b] Based on two consecutive days.

Fig. 4. LsMean ± standard errors for laser-induced fluorescence (LIF) counts across three consecutive days in the blue and red region of the visible spectrum from crushed fecal pellets extracted in chloroform ($CHCl_3$) from sheep fed a basal tobosa (*Pleuraphis mutica* Buckley) hay diet containing 0%, 10%, 20% or 30% tarbush (*Flourensia cernua* D.C.) leaves. The four diets differed ($P < 0.05$) in LIF counts across days.

the red/blue ratio, diets were similar ($P > 0.05$) across days.

Count amplitude ($P = 0.0210$) and wavelength ($P = 0.0303$) also varied in the red among collection days (Figs. 4 and 5, respectively). Profiles of the 0%, 10%, 20% and 30% diets were similar in amplitude and wavelength during the first 2 days. However, on Day 3, the amplitude and wavelength in the red decreased numerically in the 30% tarbush diet. The red/blue count ratio profile was quite similar across days for the diets containing 10% and 20% tarbush (Fig. 6). In contrast, diets containing 0% and 30% tarbush increased and decreased, respectively, ($P = 0.0259$) in their LIF count ratio on Day 3.

3.2. Intact pellets

The red/blue LIF count ratios from intact fecal pellets reflected the same trend as for crushed fecal pellets. Diets differed ($P = 0.0141$) and the ratios
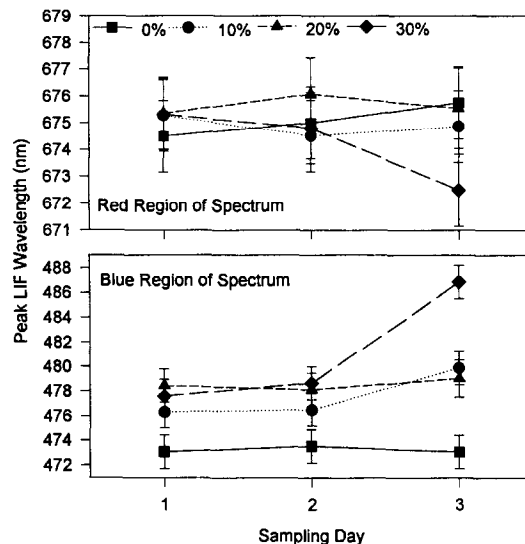

Fig. 5. LsMean ± standard errors for laser-induced fluorescence (LIF) peak wavelengths across three consecutive days in the blue and red region of the visible spectrum from crushed fecal pellets extracted in chloroform ($CHCl_3$) from sheep fed a basal tobosa (*Pleuraphis mutica* Buckley) hay diet containing 0%, 10%, 20% or 30% tarbush (*Flourensia cernua* D.C.) leaves. The four diets differed ($P < 0.05$) in peak wavelengths across days.

declined by 38% in a linear ($P = 0.0019$) fashion as tarbush in the diet increased from 0% to 30% (Fig. 3). Blue counts increased ($P = 0.0092$) across diets in a linear fashion ($P = 0.0012$; Table 1). In contrast to the crushed pellets, the blue wavelength (480 nm)
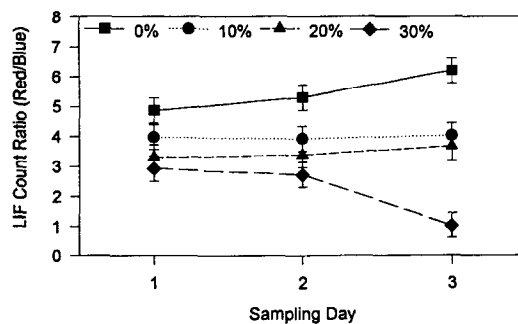

Fig. 6. LsMean ± standard errors for red to blue laser-induced fluorescence (LIF) count ratio across three consecutive days from crushed fecal pellets extracted in chloroform ($CHCl_3$) from sheep fed a basal tobosa (*Pleuraphis mutica* Buckley) hay diet containing 0%, 10%, 20% or 30% tarbush (*Flourensia cernua* D.C.) leaves. The four diets differed ($P < 0.05$) in the red/blue count ratio across days.

remained similar across diets ($P > 0.05$). Neither counts nor wavelength in the red was influenced ($P > 0.05$) by percent tarbush in the diet. In this region, the mean LIF peak occurred at 676 nm with an amplitude of 1073 counts. The blue wavelength shifted by 1 nm ($P = 0.0136$) across diets between Day 2 (481 nm) and Day 3 (480 nm). However, the day of collection did not affect ($P > 0.05$) LIF counts in the blue region of the spectrum. Likewise, day did not affect ($P > 0.05$) either LIF amplitude or peak wavelength in the red or the red/blue count ratio. In contrast to the crushed fecal pellets, the LIF from intact fecal pellets reacted similarly ($P > 0.05$) over the 2 days pellets were collected.

4. Discussion

Interpretation of LIF involves differences in number of fluorescent bands and/or relative intensity of the bands (Chappelle and Williams, 1987; Brach and Molnar, 1977). In this study, two fluorescent bands from fecal filtrate or extracts were present across diets but their relative intensities differed systematically with diet. Because the filtrate from the crushed pellets had been exposed to more fecal surface area we assumed the filtrate might contain more suspended material compared to the extract from the fecal pellets that had only been steeped but not crushed in the $CHCl_3$. However, LIF spectra for the two methods of preparation were similar. Therefore, crushing of pellets may not be required, thus reducing labor and handling. Possible differences in LIF between crushed and intact feces need to be evaluated for other diets.

Misalignment of the cuvette, underfilling of the cuvette and laser pulse-energy fluctuations may all affect intensity of the LIF signal. Therefore, red/blue ratios appear to be more reliable than measuring counts in separating tobosa diets containing differing amounts of tarbush. Even though the measured ratio depends on the spectral response of the particular spectrograph/detector equipment used, ratio trends among diets should be preserved regardless of the system used. Furthermore, by using ratios, standards were not required to calibrate the spectrograph since all samples were analyzed with one spectrograph setting.

The fluorescence observed in this study was probably from compound(s), presently unknown, rather than anions or cations, since neither the red or blue peaks were line spectra (Pringsheim, 1949). The material causing the fluorescence has several possible sources: (1) Plant material that passed through the animal's digestive tract unaltered and became concentrated in the feces; (2) Plant material that changed chemically during digestion and became concentrated in the feces; and (3) Material of animal origin (endogenous) that might have been released in response to the diets fed. Because the basal diet (tobosa hay) produced a bimodal LIF 'finger print' similar to that from all fecal pellets, it seems reasonable to speculate that the fluorescing material is of plant origin (indigestible or of low digestibility) and concentrated in the feces. Compounds causing fluorescence need not be present in high concentrations. Dyes in water at concentrations as low as 0.1 ppb have been reported to fluoresce, with a linear increase in fluorescence as dye concentration increased from 0.1 to 10 ppb (Hickman and Moore, 1970).

The tobosa fed contained substantial acid detergent insoluble nitrogen (DIN) and fiber (King et al., 1996). As tarbush in the diet increased, so did the relative magnitude of counts in the blue. This rise is probably due to the tarbush component of the diet. However, the physiological meaning of blue fluorescence from plant material is not yet understood (Rosema et al., 1991).

The excitation laser wavelength we used, 355 nm, was adequate to get blue fluorescence. However, at a laser excitation wavelength of 308 nm, in vivo discrimination of blue fluorescence from chloroplasts has not been possible (Broglia, 1993). Thus fluorescence is dependent on the excitation wavelength.

The shift observed in the blue wavelength may not be related to diet differences but rather to the method used to process the data. Had we used a smoothing function on the LIF count data, it is unlikely that the blue wavelength, especially for crushed pellet data, would have varied as much as it did.

The tarbush leaves were green but the tobosa hay was cut from standing crop composed of substantial amounts of senescent (brown and gray) material primarily composed of cell wall constituents (NDF 80%). This mixed color pattern resulting from vari-

ous plant pigments may have produced the blue LIF. Etiolated leaves with a very low chlorophyll content have shown strong blue florescence and green wheat leaves exhibit broad reflectance spectra in the blue region between 400 and 500 nm (Lichtenthaler et al., 1993).

Plants have a constituency of compounds that fluoresce when excited at the proper wavelength (Chappelle and Williams, 1987). Most of the past LIF plant related research has dealt with plant pigments in living tissue, such as the chlorophylls. Since plant pigments are extremely labile and are decomposed by acids, alkalies, oxidizing agents, hydrolytic enzymes, oxidative enzymes and strong adsorbents (Strain and Svec, 1966) it seems unlikely that the fluorescence observed from fecal material results from the same quality and/or quantity of pigments found in growing plants. Furthermore, Chappelle and Williams (1987) noted that solvent may affect fluorescence maxima of pigments, especially chlorophyll *a*. When chlorophyll *a* is suspended in acetone, its fluorescence occurs at shorter wavelengths than would be seen in its natural cellular matrix. Tannic acid, vitamin K_1 and plastoquinone all fluoresce in the vicinity of 440 nm (Chappelle and Williams, 1987). This blue wavelength was shorter than those observed for tobosa hay, tarbush leaves and fecal pellets. LIF at 470 nm has been attributed to beta carotene (Chappelle et al., 1990). This wavelength too was also less but closer (≤ 10 nm) to the mean wavelength shown by the fecal pellets used in this study. We did not determine the concentration of our samples, and this can affect fluorescence.

Lichtenthaler et al. (1993) state that fluorescence emission is reduced with increasing pigment content due to reabsorption processes, and that UV-radiation at 355 nm can barely pass the epidermis to excite chlorophyll to fluoresce. Lichtenthaler et al. (1993) determined that the best wavelength range for a pulsed UV-laser as an excitation source for LIF from vegetation is between 370 and 395 nm which was longer than the wavelength we used (355 nm) to excite our samples. Furthermore, Lichtenthaler et al. (1993) noted that longer wavelengths should be avoided since they would overlap with the resulting fluorescence.

The tarbush and tobosa plants used to formulate these diets were collected from two different sites within a 15 and 2 day period, respectively; therefore, more data are needed to account for seasonal and site differences in plant chemistry within tarbush and tobosa. Future research should concentrate on multiple-band fluorescence spectra, the development of appropriate algorithms, and the possible use of multiple-wavelength excitation sources to produce greater resolution (Brach and Molnar, 1977). Compound diets composed of more than two species must be evaluated to determine how robust the LIF procedure is in discriminating between species. The number and relative concentrations of compounds capable of fluorescence most likely will vary among plant species; therefore, differentiation and identification may result from spectral differences (Chappelle and Williams, 1987).

5. Conclusions

As tarbush in the diet increased, the red to blue LIF intensity ratio decreased linearly among diets containing 0 to 30% tarbush for both crushed and intact sheep fecal pellets. Fluorescence signatures varied among diets, with larger differences in LIF intensities (counts) and peak emission wavelength occurring in the blue compared to the red regions of the spectrum. Variability in LIF spectra, especially in the blue region of the spectrum, due to diet composition, day, and time of day will require further investigation. In addition, further research will be needed to determine the compound(s) responsible for the fluorescent signatures observed.

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